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RECOVERY OF MONKEYS AFTER MYOCARDIAL INFARCTION WITH VENTRICULAR FIBRILLATION. EFFECTS OF PGB$_x$

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• *PGB$_x$, a polymeric, stable, free radical derivative of 15-keto-prostaglandin B$_1$, that conserves oxidative phosphorylation in mitochondria under degenerative conditions* in vitro, *affected survival of male Rhesus monkeys (5-9 kg) anesthetized with pentobarbital and subjected to coronary ligation and induced ventricular fibrillation (VF). In tests performed in sequence with intervening periods for recovery, intracardiac injections of norepinephrine (NE), cardiac massage (CM), and electrical defibrillation (EDF) were used to restore cardiac function both in controls and experimental animals, but the latter were injected also with 1 mg/kg PGB$_x$. Recovery was established by maintenance of effective blood pressure without exogenous support. In the control group the cumulative survival for fibrillation episodes of 4, 6, 8, and 12 min was 60, 40, 31, and 25% respectively. In the PGB$_x$-treated group survival for equivalent periods was 100, 93, 93, and 88% respectively. In separate studies, African Green monkeys were subjected to single episodes of VF of either 8 or 12 min. Combined survival was 36% for the controls, 93% for the PGB$_x$-treated animals. Clearly PGB$_x$ radically improved cardiac recovery after circulatory arrest due to VF in the presence of acute myocardial infarction. The results also suggest a synergistic action between norepinephrine and PGB$_x$ in achieving such recovery.*

INTRODUCTION

Recovery following ventricular fibrillation (VF) in the presence of acute myocardial infarction is a well-recognized clinical problem, currently with no adequate effective therapy and a poor prognosis. Available evidence indicates that tissue ischemia leading to hypoxia and acidosis is associated with biochemical abnormalities involving insufficient energy for cellular survival because of decreased ATP production, mitochondrial damage, changes in membrane permeability, and lysosomal membrane rupture leading to irreversible cell damage and finally death.[1] Myocardial cell death is believed to occur when intracellular ATP drops below 2.0 μ moles/g in the perfused heart and the anaerobic metabolism of the ischemic heart cell stops.[2] In studies on ischemic anoxia produced by acceleration of rats at 20*g*, survival was markedly enhanced by drastic changes in pituitary-adrenal hormones which correlated with the maintenance of high levels of ATP in the brain.[3,4] In subsequent studies on degenerative biochemical changes in anoxic stress[5,6] a new bioregulatory factor was discovered with the unique property of conserving the mechanisms of oxidative phosphorylation of isolated mitochondria *in vitro* under degenerative conditions leading to a complete loss of the oxidative energy transformation process.[7,8] This factor, currently termed PGB$_x$, is a polymeric condensation of prostaglandin B$_1$ to form a new compound lacking the described properties of the parent prostaglandin.

When rat liver mitochondria are slowly

degenerated by aging at 0°C, followed by a brief Mg^{2+} catalyzed degeneration at 27°, complete loss of oxidative phosphorylation activity occurs. Addition of PGB_x to the reaction mixture preserves and restores oxidative phosphorylation to normal levels.[7,8]

In studies with inhibitors or Ca^{2+} competing for phosphorylation sites on the mitochondria, PGB_x acted to sustain oxidative phosphorylation. In the interplay between Ca^{2+} and PGB_x an *in vitro* control of the phosphorylation level could be achieved. All the effects of PGB_x were observed with so-called damaged mitochondria. No effect of PGB_x was observed with normal intact mitochondria. These findings suggested the use of PGB_x *in vivo* for the amelioration and survival of cellular catastrophes involving mitochondrial damage resulting in shock and death as seen in ischemic anoxia pathology.

The intent of the experimental design to be described was to investigate possible effect of PGB_x in the restoration of tissue and organ function after lethal periods of ischemia and hypoxia had rendered the organ intractable to the most effective therapeutic procedures known. Based on preliminary studies, the experimental procedure involved evaluation of overall cardiovascular recovery and survival of monkeys after a period of ventricular fibrillation in a heart with a left ventricular infarction from a coronary ligation. This provided an insult associated with a high incidence of mortality[9,10] and of such magnitude that recovery in untreated animals is at best difficult.

METHODS

Two species of monkeys, Rhesus (*Macaca mulata*) and African Green (*Cercopithecus aethiops*), were used. The monkeys were anesthetized with pentobarbital (30 mg/kg), then a thorocotomy on the left side between the 4th and 5th intercostal space was performed under positive pressure artificial respiration. Catheters were placed in the thoracic or abdominal aorta for direct recording of blood pressure and heart rate, and in the vena cava for venocylsis with Normasol, pH 7.4.* Lead I ECG was obtained with intradermal electrodes. Two stainless steel EEG electrodes were anchored 3 cm apart into the skull along the temporal ridge, positioned not to penetrate the dura. After stabilization, so that blood pressure was constant and the animal was able to maintain itself without assistance, the left anterior interventricular coronary artery was ligated just past the major branch approximately 1 cm from origin. In separate studies on Rhesus monkeys this ligation procedure caused an ischemic region involving an average of 27% of the left ventricular mass as measured by the radioactive microsphere technique.[11]

Coronary occlusion was utilized as part of the experimental design to assure that fibrillation once induced would be maintained. In initial studies in which VF was induced in animals without coronary occlusion there had been a high incidence of spontaneous defibrillation. Furthermore, the overall recovery after short periods of VF (4 or 8 min) was too high to permit an efficient assessment of any protective effect.

Arterial blood pressure, EEG, ECG, and heart rate were recorded for all animals. Myocardial segment tension, intraventricular pressure, dp/dt, and end-diastolic pressure as well as arterial blood gases, pH, and blood glucose levels were measured in selected animals. Since this latter information afforded little data correlative with recovery or death of the animal, it will not be referred to further in this paper.

Following coronary ligation, VF occurred

* Normasol R, pH 7.4, a preparation marketed by Abbott Laboratories, contains sodium chloride, 0.526%; sodium acetate, 0.222%; sodium gluconate, 0.52%; potassium chloride, 0.037%; and magnesium chloride, 0.014%.

spontaneously in approximately half the animals in the first 10 to 20 min. Incidence of spontaneous VF prior to 20 min was 56% in the controls and 44% in the PGB_x-treated group. The difference is not statistically significant. In those animals that did not fibrillate within the 20-min period after ligation, VF was induced electrically. VF was permitted to continue for specified time periods ranging from 4 to 24 min.

At the end of the prescribed period, resuscitation procedures were started consisting of (a) intracardiac injection of 500 μg norepinephrine (NE), (b) cardiac massage, and (c) electrical defibrillation. The latter was achieved with a DC defibrillator set to deliver 50-W pulses for 0.15 sec. The PGB_x-treated monkeys received the same resuscitation regime as the controls but with the additional intracardiac injection of 1 mg/kg PGB_x followed by cardiac massage and electrical defibrillation. All intracardiac injections were made into the left ventricular cavity. In addition, the treated animals received PGB_x (1 mg/kg) intramuscularly just prior to coronary ligation as well as additional doses of PGB_x (1 mg/kg) intravenously every 30 min throughout the experimental periods. PGB_x, prepared from 15-diketo PGB_1 methyl ester according to the method of Polis et al.[12] was administered as the sodium salt dissolved in Normasol R, pH 7.4, to a concentration of 10 mg/ml just before use.

Once the electrical and contractile activities of the heart were reestablished, the animal was allowed to recover spontaneously. If the animal remained in shock, NE (1-10 μg) was infused intravenously until the animal attained a blood pressure level over 40/20 mm Hg or became refractory to NE and died. If the monkey recovered from the first 4 min of fibrillation and became stable for a period of 20 to 30 min, it was subjected to the next longer fibrillation period of 6 min. In this manner animals were subjected sequentially to episodes of fibrillation of 4, 6, 8, and 12 min duration separated by 20-30 min recovery periods until the animal died in shock or successfully survived the course. The last group of animals, after recovering from 12 min of VF, was subjected to 24 min of VF.

Paired control and treated monkeys were run on the same day. When a control was run in the morning of one day and a treated animal in the afternoon, the order was reversed with the next pair. As far as possible, selection of the animals was random. In six instances, control monkeys that could not be brought out of shock with NE were then given PGB_x intravenously. Four out of six animals so treated revived to survive the sequential fibrillation series to 24 min.

To permit evaluation of the cardiovascular shock or recovery with PGB_x over a period of time after one ischemic event, another series of studies was made with African Green monkeys subjected to a single fibrillation episode of either 8 or 12 min. Recovery procedures were the same as with the sequential fibrillation studies except that the blood pressure levels in those animals that survived the initial fibrillation period were monitored for at least two hours. In general those animals that failed in shock did so within the first hour after defibrillation. This also permitted the determination of the ability of NE to maintain blood pressure levels in the presence or absence of PGB_x and thus evaluate the synergistic effects of PGB_x and NE.

For the *in vitro* studies, rat liver mitochondria were isolated by differential centrifugation in 0.3 M sucrose containing 5×10^{-4} M EDTA pH 7.4. The isolation and assay methods employed standard techniques.[13] Mitochondrial preparations used contained little lysosomal activity as evidenced by measurements of acid phosphatase activity, which was less than 2% of that found in whole liver.[14] Addition of PGB_x

did not alter the acid phosphatase activity in these preparations. Mitochondria were stored in 0.3 M sucrose containing 5×10^{-4} M EDTA at a concentration of 100 mg protein/ml at 0°C until used. Although the PGB_x effect on damaged mitochondria could be shown with first day preparations, 3-5 days of aging at 0°C normally was required to demonstrate the maximum PGB_x effect. On the other hand, some rat liver mitochondria preparations were found to be active even after 10 days at 0°C. For the electron microscopy studies of freshly isolated rat liver mitochondria, samples were centrifuged at 6000*g*, the sucrose removed and the pellet layered with 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. At 30-min intervals the mixing reagent was changed 3 times. For the control and PGB_x experiments, 4 mg of 5-day-old mitochondria were incubated in a mixture containing 0.1 ml of 0.1 M potassium phosphate buffer, pH 7.4; 0.16 ml of 0.20 M sodium α-ketoglutarate, pH 7.4; and 0.1 ml of 0.1 M $MgSO_4$, in a total vol of 2.01 ml for 15 min in a shaker bath at 27°C. In the PGB_x experiments 10 μg of PGB_x was added to the above mixture. At the end of the preincubation period, the assay for phosphorylation was begun by the addition of 0.15 ml of a mixture of 0.05 ml of 0.1 M ADP, 0.05 ml of 0.1 M AMP, and 0.05 ml of 2 M KCl, followed immediately by the addition of 0.04 ml of 3.75% solution of crystalline bovine serum albumin to give a final mixture of 2.2 ml. AMP and ADP were added as phosphate acceptors, KCl was added to maintain tonicity, and serum albumin for

TABLE I. Survival of Control and PGB_x-Treated Monkeys Subjected to Incremental Periods of Fibrillation after Left Anterior Coronary Artery Ligation

Control							PGB_x-treated						
No.[a]	Fibrillation (min)						No.	Fibrillation (min)					
	4	*6*	*8*	*12*	*24*			*4*	*6*	*8*	*12*	*24*	
18 C	F[b]						14 E		F				
31 C	F						15 E		S	S	S		
35 C	F						19 E	S	S		S		
37 C	F						20 E	S	S	S	S		
32 C	S[c]	F					21 E	S	S	S	S		
33 C	S	S	F				22 E	S	S	S	S		
24 C	S	S	S	S			23 E	S	S	S	S		
45 C	S		F				36 E	S	S	S	F		
11 C		F					38 E	S	S	S	S	S	
12 C		F					39 E	S	S	S	S	F	
13 C		S	S	S			40 E	S	S	S	S	S	
16 C		S	S	F			41 E	S	S	S	S	S	
17 C		F					42 E	S		S	S	S	
26 C		F					43 E	S		S	S	S	
25 C	S	S	S	S	F		27 E	S	S	S	S		
44 C	S		S	S	S		29 E	S	S	S	S		
	Cumulative % survival												
	60	40	31	25				100	93	93	88		

[a] Refers to identification number of individual monkey. [b] (F) indicates monkey failed to recover from the fibrillation and sustain a blood pressure above shock. [c] (S) indicates successful recovery out of shock for the trial period.

binding fatty acids released during the degeneration of the mitochondria. This sequence for the addition of reactants was found to be optimal for demonstrating the PGB_x effect.

In order to obtain enough material for the EM experiment, five control vessels and 5 vessels containing PGB_x were reacted with constant shaking for 20 min. The reaction vessels were pooled in ice-cold tubes and centrifuged at 10,000*g* for 10 min. The supernatants were removed and analyzed for inorganic phosphate remaining[15] in order to check the effectiveness of the PGB_x preparation. The pellets were then fixed with cold buffered glutaraldehyde as described before.

The fixed pellets from the freshly isolated mitochondria from control and PGB_x reacted mitochondria were minced with a freshly degreased razor blade, and the resulting segments were immersed in Millonigs-phosphate buffer (MPB) and fixed further with 1.0% OsO_4 in the MPB for one hour. Fixation was followed by rinsing in several changes of MPB, and dehydration was accomplished in a graded series of alcohol solutions (30, 60, 90, 100, and again 100%) followed by 2 changes in propylene oxide, impregnated and embedded in epoxy. Thin sections of the epoxy-embedded mitochondria, cut with a Sorval MT2B ultramicrotome fitted with a diamond knife, were post-stained with uranyl acetate and lead citrate.

For the electron microscopic studies of monkey heart tissue, the aorta was cannulated and the beating heart was subjected to retrograde perfusion at a pressure of 110 cm H_2O initially with buffered saline and subsequently to 1.25% glutaraldehyde buffered with 0.08 M sodium cacodylate and 0.03 M $CaCl_2$ (pH 7.4).[16] Small tissue samples were obtained from normal and ischemic regions; they were immersed in the same fixative for one-half hour. Tissues were further trimmed to segments measuring about 0.5 mm^3 and post-fixed in 1.0% OsO_4 (0.1 M cacodylate with 3.0% sucrose) at 4°C and brought to room temperature for a total post-fixation period of one hour. The tissue, after being rinsed twice in 0.2 M cacodylate buffer dehydrated in a graded series of alcohol solutions, was treated with propylene glycol prior to embedding in epoxy as described above. For each study, 9 electron images of each of 6 examples were recorded to electron optical magnifications of 3400× and 8200× employing an RCA EMU-4 electron microscope. All the EM observations and interpretations were made by Dr. John T. Stasny.

RESULTS

Studies in Rhesus Monkeys

Initial fibrillation. Table I summarizes the results of the survival of Rhesus monkeys subjected to periods of fibrillation. A total of 10 control and 14 PGB_x-treated animals were subjected to an initial episode of VF of 4-min duration. At the end of this period, 6 of the controls and all 14 of the PGB_x-treated animals recovered. This difference is statistically significant at $p < 0.02$ (Fisher's exact test).

Sequential fibrillation. Since all of the PGB_x-treated animals survived the initial tests of 4 min, the studies were extended by subjecting all survivors (control and treated) to additional periods of VF. After the initial episode, animals were allowed to recover and were subjected to progressively longer periods of VF of 6, 8, 12, and 24 min. As shown in Table I, the cumulative survival in the controls decreased from 60% at 4 min to 25% after the 12-min episode. In the PGB_x-treated group, the 100% survival rate after 4 min of VF was maintained at 88% after the sequential exposure to 6, 8, and 12 min of VF. This difference is sta-

tistically significant at $p < 0.01$ (chi-square test). Furthermore, these results indicate that while survival in the controls was decreasing progressively with the longer periods of VF as may be expected, the PGB_x-treated group maintained a high survival rate in the successive tests. This provides additional evidence that the differences obtained in the initial tests were not due to chance.

Six of the PGB_x-treated animals that survived the 12-min period were exposed to 24 min of VF. Of these, 5 survived. Of the 4 controls that survived 12 min of VF, 2 were tested at 24 min. One survived. Specific statistical comparisons of the survivors after 24 min of VF is not possible since not all the animals surviving the 12 min of VF were tested at 24 min (Table I).

Defibrillation was achieved with a single shock in most of the PGB_x-treated monkeys (12 of 14) and about half the control monkeys (6 of 10). A few controls required 2 to 3 shocks. In general, electrical defibrillation was uniformly successful and was not a significant factor in survival. The major difference between the PGB_x-treated and control animals was, that in the former the recovery of cardiac contractile force following electrical defibrillation was more rapid and more complete. Control animals in which cardiac contractile activity did not recover sufficiently to maintain an adequate level of blood pressure often developed recurrent episodes of fibrillation and/or cardiac arrest during the resuscitation phase. In general, such animals did not recover. These observations on untreated animals are similar to those observed in other species (dogs, pigs) in this and other laboratories. There was, however, a difference in the incidence of spontaneous defibrillation. Many of the PGB_x-treated animals defibrillated spontaneously and required repeated electrical interventions to maintain the fibrillation for the experimental period. This was rarely observed in the controls. Table II summarizes the frequency of spontaneous defibrillation in control and PGB_x-treated monkeys.

TABLE II. Spontaneous Recovery from Ventricular Fibrillation After Induced Myocardial Infarction in Rhesus Monkeys

Fibrillation time (min)	Incidence of spontaneous defibrillation (%)	
	Controls	PGB_x-treated
4	0	8
6	7	34
8	7	39
12	0	29
Mean:	3.7	28

Studies in African Green Monkeys

Initial VF. Studies involving a single prolonged episode of VF were made on 14 control and 14 PGB_x-treated African Green monkeys. Two different groups of animals were exposed to a single episode of either 8 or 12 min of VF. The results, summarized in Table III, were simliar to those obtained in the sequential VF studies with Rhesus.

Of those Greens exposed for 8 min, 89% of the PGB_x-treated group recovered and 33% of the controls recovered. Of those exposed for 12 min, 100% of the PGB_x-treated group recovered and 40% of the control group recovered. The differences are statistically significant ($p < 0.05$). The combined survival (8 and 12 min) was 93% for the treated as compared to 36% for the controls ($p < 0.01$).

This experimental design, involving a single episode of VF, made possible extended observation on the cardiovascular status of the animal for a period of 2-3 hours after VF. It was noted that a significant number of control animals, that had recovered cardiac activity after initial resuscitation rapidly deteriorated into a state of circulatory shock.

TABLE III. Survival of Control and PGB_x-Treated African Green Monkeys after a Single Fibrillation Episode

VF period (min)	Control		PGB_x-treated	
	No. surviving / Total tested	% Survival	No. surviving / Total tested	% Survival
8	3/9	33	8/9	89[a]
12	2/5	40	5/5	100[a]
Total	5/14	36	13/14	93[b]

[a] $p < 0.05$. [b] $p < 0.01$.

During this period, repeated administrations of NE to these controls resulted in short-lived pressor effects that became progressively less (and/or required higher doses of NE) until no effect could be obtained with even very large doses of NE. In contrast, many PGB_x-treated animals responded to NE with a pressor effect that did not return to the previous baseline. Thus in the PGB_x-treated group, progressively smaller doses of NE were needed to maintain adequate blood pressure, and eventually the pressure was maintained without any exogenous NE.

Infusions of catecholamines after resuscitation. To evaluate this phenomenon more directly, experiments were carried out in which animals were infused continuously with NE following a period of VF, and the infusion rate was adjusted to maintain a diastolic pressure of 60 mm Hg during the post-fibrillation period. The doses of NE required to achieve this ranged from 0.3 to 300 μg/min. Six control and 12 PGB_x-treated African Green monkeys were studied after the initial resuscitation. In 5 out of 6 controls (83%) the amount of NE infused had to be progressively increased to maintain the desired pressure. In 4 of these animals the pressure could not be maintained and the animals died. By contrast, in 7 out of 12 PGB_x-treated animals the amount of NE infused necessary to maintain 60 mm Hg was progressively decreased, and all 12 animals survived.

In other experiments with control animals whose blood pressure was at shock levels, the administration of repeated doses of NE did not produce a pressor effect. When these animals were treated with PGB_x, subsequent administration of NE at the previously ineffective dose levels usually produced distinct pressor responses.

Catecholamines and PGB_x. In initial studies it was found that intracardiac administration of NE during the resuscitation period improved greatly the incidence of recovery and the subsequent status of the animal in both control and PGB_x-treated preparations. Therefore intracardiac NE was included as a standard measure in the studies reported here. Observations made during these initial and subsequent studies suggested a potentiating effect between PGB_x and catecholamines. To determine whether PGB_x potentiated any of the cardiovascular effects of NE in normal animals, 3 normal anesthetized monkeys (Green) were investigated for dose-response pressor and cardio-accelerator effects of NE (2-5 μg/kg) before and after PGB_x. These did not demonstrate any potentiating effect. However, in the animals with blood pressure at shock levels, pretreatment with PGB_x produced a distinct potentiation of the pressor actions of NE. An example is shown in Fig.

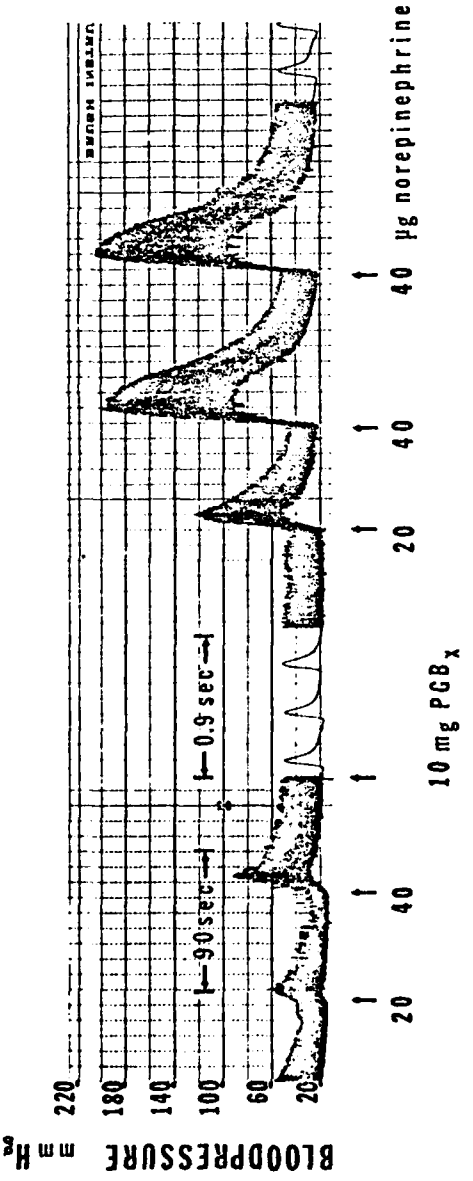


FIGURE 1. Potentiation of the pressor effect of norepinephrine (NE) following treatment with PGB_x. This animal had shock levels of pressure and negligible responses to 20 and 40 µg doses of NE. The same doses of NE produced marked pressor responses when tested within 3 to 5 min after administration of 10 mg of PGB_x. Blood pressure scale: 20 mm Hg/div. Time scale: 10 sec/div (slow record) and 0.1 sec/div (fast record).

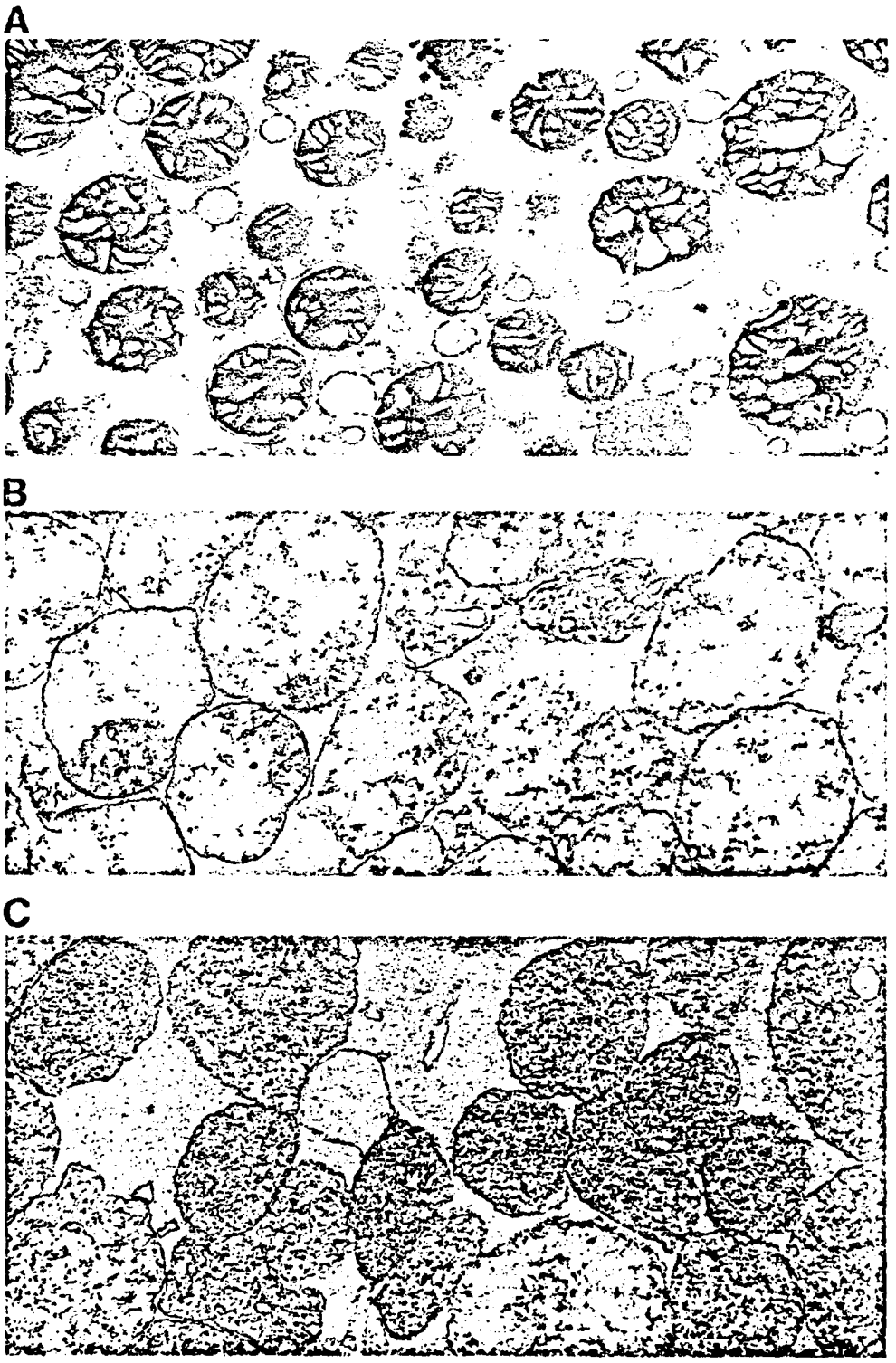
1. Unfortunately this effect was evident only in the presence of severe hypotension (below 40 mm Hg), was not consistent, and/or the conditions necessary to reproduce it could not be fully identified. Hence it was not possible to obtain quantitative information on the potentiation, nor was it feasible to study the effect of adrenergic blocking agents.

Electron Microscopic Studies

In vitro studies. In an effort to complement the kinetic studies *in vitro*, and the recovery studies *in vivo* with correlative changes in mitochondrial structure, electron microscopy was carried out on isolated mitochondria subjected to degenerative conditions in the absence and presence of PGB_x. These are compared with the sections of heart from areas considered normal and from the infarct area of control and PGB_x-treated monkeys after fibrillation for 12 min followed by the resuscitation procedures.

The EM observations made in the 3 mitochondrial preparations are illustrated in Fig. 2. Figure 2A represents normal 6-hour-old

FIGURE 2. Transmission electron microscopy observations on isolated rat liver mitochondria (20,000 ×). (A) These are 6-hour-old mitochondria of excellent homogeneity. Almost all are in state III or the condensed configuration that represents the low energy state of isolated rat liver mitochondria. The matrix material is very dense. Only a few mitochondria are not in the condensed state. Some microsomes also can be seen. (B) These are 5-day-old mitochondria isolated after preliminary degeneration and 20-min reaction as described under METHODS. All are swollen 2 to 3 times the size of the 6-hour-old mitochondria but do not appear lysed and show only a very small amount of granular intramitochondrial content and almost no remnants of cristae. (C) These are 5-day-old mitochondria treated with PGB_x and isolated after preliminary degeneration and 20-min reaction as described under METHODS. The PGB_x-treated mitochondria are less swollen than the untreated mitochondria (B) and contain more matrix material and membranous derivatives of cristae.


A
B
C

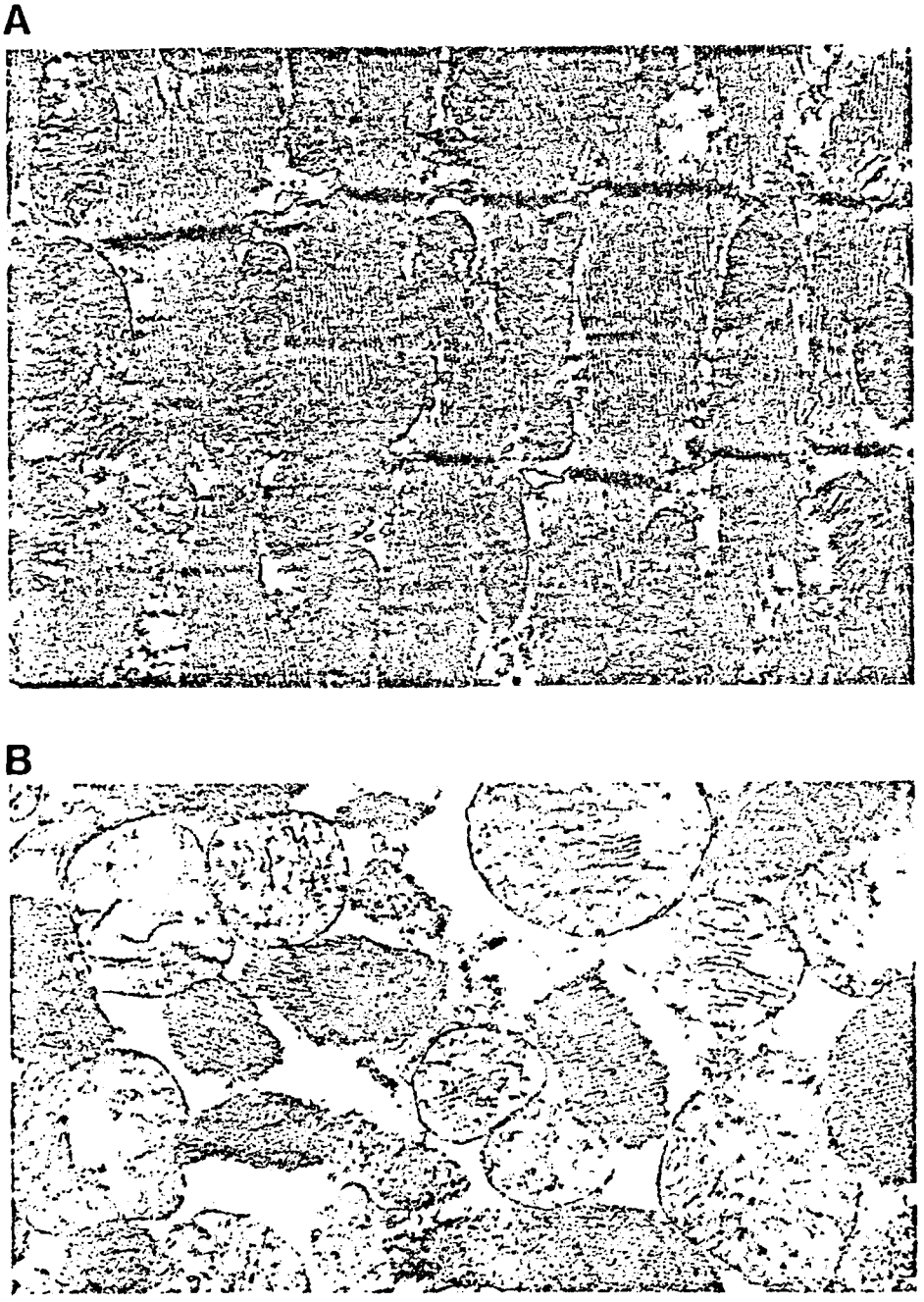


FIGURE 3. Transmission electron microscopy observations on tissue sections from an untreated monkey with myocardial infarction after 12 min of VF (24,600 ×). (A) Noninfarcted tissue from base of left ventricle. In noninfarcted region, mitochondria in some areas are well preserved while in other areas they are lacking in matrix density but show numerous intact and prominant cristae. (B) Center of infarcted area. Mitochondria are swollen, disorganized, and deteriorated. Cristae are in short segments within a leached matrix.

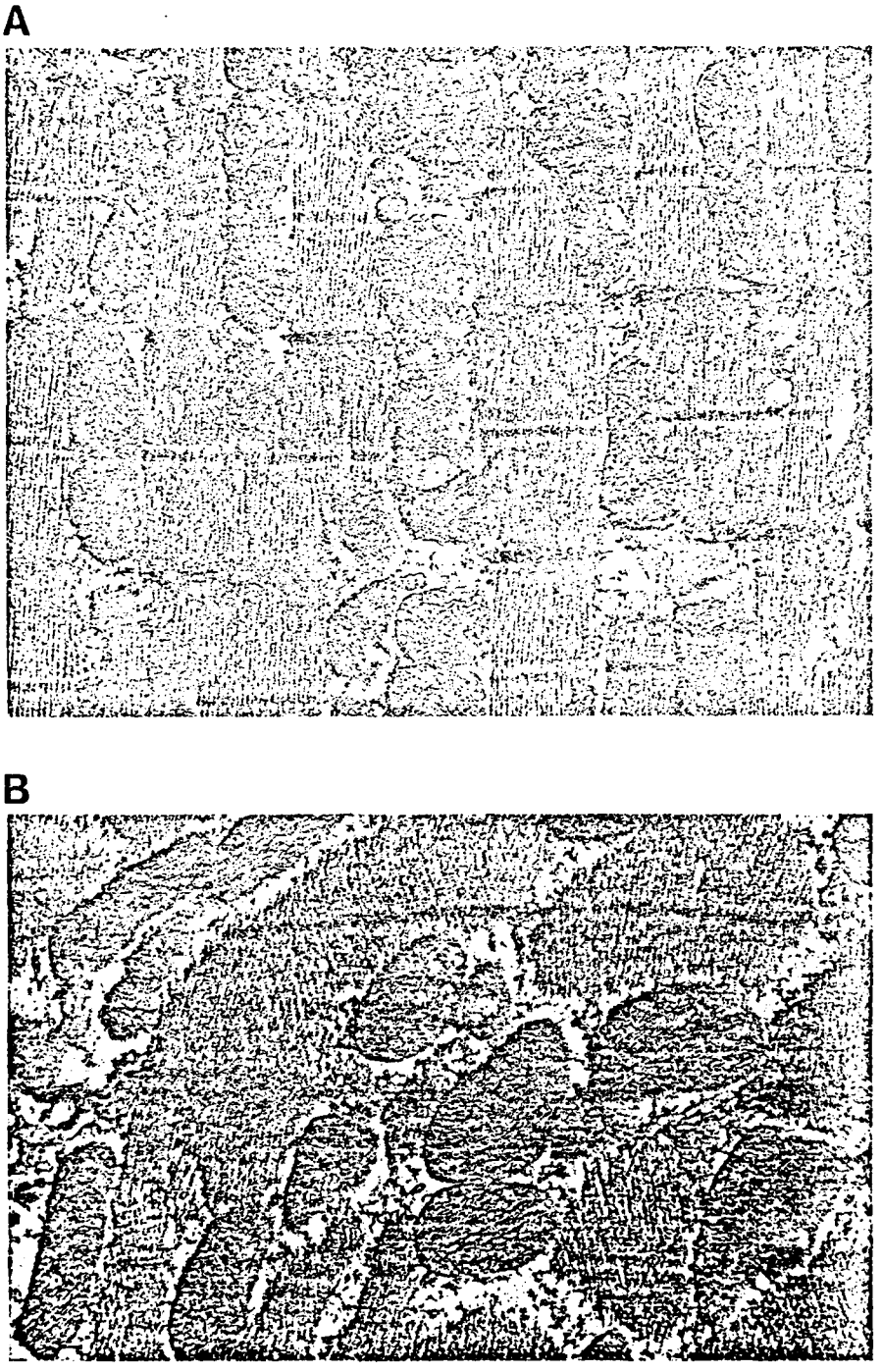


FIGURE 4. Transmission electron microscopy observations on tissue sections from a monkey treated with PGB, after 12 min of VF (24,600 ×). (A) Noninfarcted tissue from base of left ventricle. Mitochondria matrix in general is dense and in good condition. (B) Center of infarcted area. Mitochondria are numerous and small; some show unusual shapes but are nevertheless intact and dense.

mitochondria showing the intact and homogenous condition of the original isolated preparation. This is consistent with the high phosphorylation ability of this preparation (6 μmoles of inorganic phosphate esterified per 4 mg mitochondria for the 20-min reaction period). Figure 2B shows mitochondria degenerated for 15 min and reacted under conditions of oxidative phosphorylation. This preparation, which esterified only 0.32 μmoles of inorganic phosphate, served as the control. The mitochondria in the EM of Fig. 2C are similar to the control (2B) except that 10 μg of PGB_x were added to the reaction mix. In the presence of PGB_x, oxidative phosphorylation was conserved and the mitochondria esterified 4.36 μmoles of inorganic phosphate over the same time which is equivalent to 73% of the maximum phosphorylation obtained with freshly isolated mitochondria. Details of the EM differences are outlined in the legend of Fig. 2.

In vivo studies. Tissues for the electron microscopic studies were removed from 3 untreated and 3 PGB_x-treated monkeys. In these studies the animals were subjected to single periods of VF of 12 min and in all cases VF was induced 20 min after ligation.

Sections were taken from the left ventricle, thus: (a) from a distant, apparently undamaged area in the base of the left ventricle and (b) from the middle of the infarcted region. Representative sections are shown in Figs. 3 and 4. Tissues shown in Figs. 3A and 3B were taken from an untreated monkey after a 12-min period of VF once it was established that the animal had failed to recover. The PGB_x-treated animal (Figs. 4A and 4B) was also exposed to 12 min of VF, but it survived and was sacrificed after a 2-hour monitoring period. In general, the mitochondrial tissues from PGB_x-treated animals were in good condition. Although many mitochondria in the infarcted area show bizarre elongated shapes, their matrix density and the good condition of membranes suggest structural integrity. This differs considerably from the degenerated, vacuolated condition of the mitochondria from equivalent regions of the untreated animal hearts (Fig. 3B). Tissue samples from the "border" zone were also studied. These showed similar differences between control and treated animals. In view of this, and considering the ambiguities of sampling from the border zone, these studies did not provide any additional information and are not included in this report.

For our purposes morphometric considerations were of less concern than the condition of the mitochondria, the organelles targeted for demonstrating the response to treatment. Hence quantitative data were not recorded. Similarly, no attempt was made to preferentially orient the tissue fibers during preparation for EM to show sarcomeres in comparable displays. The fixation procedure used was that of Tomanek and Karlsson,[16] which is considered to be optimal. However, it should be emphasized that all the tissues studied, including those obtained from non-ischemic regions, were subjected to periods of ischemia and hypoxia during the periods of induced VF. Alterations in tissue structure would therefore be expected. In view of this, the preserved state of the mitochondria of the treated animals is to be considered remarkable.

DISCUSSION

The primary hypothesis underlying the described experiments is that since PGB_x has a unique *in vitro* action in the conservation of oxidative phosphorylation under conditions degenerative to mitochondria, a similar action *in vivo* should serve to enhance survival of an ischemic anoxic crisis incurring mitochondrial damage. This implies that car-

diac muscle contraction is limited by the rate at which chemical energy can be supplied by the metabolic process. Although there is ample evidence[1] for biochemical and morphological changes in the infarcted myocardium with considerable damage in the forms of vacuolation, swelling, and loss of structure seen in mitochondria, it is important to remember that a distinction exists between biochemical and structural damage. Mitochondria that may take a long time to recover morphologically seem to regain and maintain their functional integrity even though they appear ragged and disrupted.[17] Despite these elements of uncertainty for specific sites of dysfunction, there remains the overall failure in the mechanism coupling energy transformation with energy utilization. It is in this mechanism that we propose a role for PGB_x, which reacts synergistically with norepinephrine to reestablish the flow of energy to the contractile process in cardiogenic shock. PGB_x can be definitively associated with conservation and reactivation of mitochondrial synthesis of ATP from the *in vitro* findings.[7,8] According to Ellis,[18] the evidence for norepinephrine action on contraction seems to be that it takes place at some site coupling metabolic energy to the contractile process. Catecholamines simultaneously increase ATP breakdown and contractile force.[19] Adrenergic mediators also increase the maximum velocity of shortening.[20,21] The combined action of PGB_x and norepinephrine then could reestablish both sufficient energy and sufficient utilization to account for survival after the ischemic crisis.

The repeatedly confirmed findings that PGB_x has no effect or even a small inhibitory action in intact mitochondria *in vitro* and that it has no demonstrable effect on the normal animal cannot be overemphasized. The dramatic actions of PGB_x occur only with damaged mitochondria *in vitro* or after ischemic pathology *in vivo*. The effects observed suggest the PGB_x can replace or bridge an essential factor in energy transformation that is lost when mitochondria are swollen and damaged.

The results obtained in the studies of both Rhesus and African Green monkeys indicate that treatment with PGB_x greatly improves the incidence of survival after periods of complete circulatory arrest produced by VF. This conclusion can be based primarily on the results attained with the initial tests of VF used in Rhesus monkeys and with the single spisodes of VF used in African Green monkeys. Considering both species, the survival after single or initial episodes of VF of 4, 8, and 12 min was 60%, 32%, and 29% in the controls and 100%, 92%, and 90% in the PGB_x-treated. Studies with the repeated sequential VFs performed in the Rhesus monkeys provide additional confirmation that the differences in the initial episodes were not due to a chance occurrence, even at a low probability. If the higher survival to the first test were due to a chance occurrence, survivals to subsequent progressively longer results would be expected statistically to show an exponentially decreasing rate of cumulative survival, as was found in the case of the controls. By contrast, the PGB_x-treated group has a relatively unchanged rate of survival over the period tested, providing strong statistical evidence that the initial result obtained was not due to a chance occurrence. The cumulative test results also show that the median survival time (for 50% survival) after VF was increased from between 4 to 6 min in the controls to more than 12 min in the PGB_x-treated animals. Recovery after circulatory arrest depends initially on the restoration of electrical and mechanical activity of the heart and subsequently on the reestablishment of the cardiovascular control mechanisms responsible for the maintenance of effective blood pressure levels. Improved

recovery is therefore likely to involve primarily cardiac effects followed by reversal of the shock state.

The experimental methods used in the present studies included prior insult to the heart by coronary ligation involving a significant proportion of the left ventricle. Under these conditions improved cardiac resuscitation could be the result of improvements of (a) the general status of the entire heart, or (b) primarily the marginal ischemic regions.

It is well known that cardiac resuscitation is more difficult in the presence of coronary occlusion.[9,10] Therefore one possible interpretation of the results is that treatment with PGB_x in some way alters the degree of myocardial injury associated with coronary occlusion. This could be the case if PGB_x reduced the size of the metabolically injured myocardium following coronary occlusion. It is now generally believed that tissue injury produced by coronary occlusion includes a significant portion of marginal tissue with diminished blood flow, the ultimate fate of which depends upon the discrepancy between the tissue metabolic demands and the reduced circulation.[22-24] The effect of PGB_x in restoring phosphorylating activity of previously damaged mitochondria *in vitro* is consistent with the possibility that the results of the present study could be due to PGB_x actions on the marginal areas of coronary occlusion. However, considering that VF produces generalized hypoxia in the entire heart, an equally likely action of PGB_x is that it affects the ability of the entire heart to recover. Most likely, both factors play a significant role in determining the difference in the survival between control and PGB_x-treated animals in the present experiments.

Alternative interpretations of the results also should be considered. It is possible that the observed effect of PGB_x in the intact animal is unrelated to its actions on isolated mitochondria and that it represents unrecognized effects on the sarcoplasmic reticulum or other cellular membranes such as lysosomes. Moreover the *in vitro* effect of PGB_x in favoring phosphorylation to Ca^{2+} uptake may have its counterpart in regulating excess Ca^{2+} in the contractile process of the anoxic myocardium. It is also possible that the observed *in vivo* effect may depend on the interaction between PGB_x and other naturally occurring compounds. In this connection the potential interaction between PGB_x and circulating catecholamines is of particular interest in view of the observations made during the present studies on the biological interactions between PGB_x and norepinephrine. All these alternates represent plausible speculations subordinate to the firm experimental evidence of PGB_x action on mitochondria.

Although PGB_x is a polymeric derivative of prostaglandin B_1, it exhibits none of the reported activities of any of the known prostaglandins. Both its molecular size and structure favor a unique metabolic action not shown by the monomeric prostaglandins. It is also unrealistic to expect that PGB_x would be converted metabolically to a monomeric prostaglandin. Therefore it would be unlikely to have biological properties attributed to the known prostaglandins. PGB_x has no structural similarity to PGX (PGI_2 or prostacyclin) recently identified.[25,26] It should be noted that PGB_x has none of the cardiovascular actions reported for prostacyclin, such as systemic vasodilation and reduction of blood pressure, nor is there any evidence that PGB_x shares any of the cellular or antithrombotic effects of prostacyclin. Therefore the suggested actions of prostacyclin on myocardial infarction[27] may be basically quite distinct from those described here for PGB_x.

Similarly, recent reports on the effects of monomeric prostaglandins on the ischemic myocardium[28-30] appear to involve a different mechanism of action than that of

PGB_x. Furthermore, none of the single prostaglandins have an effect on mitochondrial phosphorylation *in vitro* comparable to that of PGB_x. On the other hand, it is possible that the PGB_x effects *in vivo* may be related to some as yet unidentified activity perhaps shared to a greater or lesser extent by other prostaglandins. In any event, more recent studies with PGB_x in other experimental preparations involving tissue ischemia *in vivo* have confirmed a beneficial effect of this compound in promoting subsequent recovery.[31,32]

In general the present results favor the view that PGB_x has an activity *in vivo* similar to that previously demonstrated in isolated mitochondria. PGB_x then would constitute the prototype of an entirely new class of compounds whose biological activity would involve restoration of metabolic functions following hypoxic or ischemic injury. Pharmacological compounds possessing such an activity would have a broad application in a variety of diseases and traumatic states.

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REFERENCES

1. B. E. Sobel. Biochemical and morphologic changes in infarcting myocardium. In *The Myocardium: Failure and Infarction*. F. Braunwald. Ed. H. P. Publishing, New York, 1974, pp. 247–260.
2. W. Kubler and P. G. Spieckerman. Regulation of glycolysis in the ischemic and anoxic myocardium. *J. Mol. Cell. Cardiol., 1,* 351 (1970).
3. B. D. Polis. Hormonal determinants of mammalian tolerance to acceleration stress. *J. Appl. Physiol., 16,* 211 (1961).
4. H. W. Shmukler, B. D. Polis, and J. Wyeth. Variations in brain nucleotides in anoxic stress. In *41st National Aerospace Medical Association Conference,* St. Louis, Missouri, 1970.
5. B. D. Polis, R. P. Miller, and A. M. Grandizio. Prostaglandin induced, stress related, phospholipid changes in blood and brain. *Physiol. Chem. Phys., 6,* 287 (1974).
6. B. D. Polis, E. Polis, J. DeCani, H. P. Schwarz, and L. Dreisbach. Effects of physical and psychic stress on phosphatidylglycerol and related phospholipids. *Biochem. Med., 2,* 286 (1969).
7. B. D. Polis, A. M. Grandizio, and E. Polis. Some *in-vitro and in-vivo* effects of a new prostaglandin derivative. *Adv. Exp. Med. Biol., 33,* 213 (1973).
8. B. D. Polis, E. Polis, and S. Kwong. Protection and reactivation of oxidative phosphorylation in mitochondria by a stable free radical prostaglandin polymer (PGB_x). *Proc. Natl. Acad. Sci. USA, 76,* 1598 (1979).
9. E. F. Wiggers. The physiologic basis for cardio-resuscitation from ventricular fibrillation. Method for serial defibrillation. *Am. Heart J., 20,* 413 (1940).
10. C. K. Friedberg. *Diseases of the Heart.* W. B. Saunders, Philadelphia, 1966, pp. 573–575.
11. E. T. Angelakos, L. Carballo, A. Sternberg, and J. Carballo. Distribution of microspheres in monkey hearts and determination of non-perfused myocardial weight after coronary ligation. *Fed. Proc., 34,* 722 (1975).
12. B. D. Polis, S. Kwong, E. Polis, G. Nelson, and H. W. Shmukler. Studies on PGB_x, a polymeric derivative of prostaglandin B_1: I. Synthesis and purification of PGB_x. *Physiol. Chem. Phys., 11,* 109 (1979).
13. G. H. Hogeboom, W. C. Schneider, and G. E. Pallade. Cytochemical studies of mammalian tissues. I. Isolation of intact mitochondria from rat liver; some biochemical properties of mitochondria and submicroscopic particulate material. *J. Biol. Chem., 172,* 619 (1948).
14. E. Reid. Preparation of lysosome-rich fractions with or without peroxisomes. In *Subcellular Components.* G. D. Birnie, Ed. University Park Press, Baltimore, 1972, pp. 93–118.
15. R. H. Dreisbach. Submicrogram determination

of inorganic phosphate in biological material. *Ann. Biochem., 10,* 169 (1965).
16. R. P. Tomanek and U. L. Karlsson. Myocardial ultrastructure of young and senescent rats. *J. Ultrastruct. Res., 42,* 201 (1973).
17. A. Schwartz, J. M. Wood, J. C. Allen, E. P. Bornet, M. L. Entman, M. A. Goldstein, L. A. Sordahl, and M. Suzuki. Biochemical and morphological correlates of cardiac ischemia. *Am. J. Cardiol., 32,* 46 (1973).
18. S. Ellis. Catecholamines and contraction. In *Factors Influencing Myocardial Contractility.* R. D. Tanz, F. Kavaler, and J. Roberts, Eds. Academic Press, New York, 1967, pp. 437–442.
19. J. R. Williamson and D. Jamieson. Dissociation of the inotropic from the glycogenolytic effect of epinephrine in the isolated rat heart. *Nature, 206,* 364 (1965).
20. E. H. Sonnenblick and W. W. Parmley. Active state in heart muscles; force-velocity-length relations, and the variable onset and duration of maximum active state. See ref. 18, pp. 65–83.
21. A. J. Brady. Physiological appraisal of the action of catecholamines on myocardial contractions. *Ann. NY Acad. Sci., 139,* 661 (1967).
22. P. R. Maroko, J. K. Kjekshus, B. E. Sobel, T. Watanabe, J. W. Covell, J. Ross, and E. Braunwald. Factors influencing infarct size following experimental coronary artery occlusions. *Circulation, 43,* 67 (1971).
23. P. R. Maroko and E. Braunwald. Effects of metabolic and pharmacologic interventions on myocardial infarct size following coronary occlusion. *Ibid., 53 (Suppl. I),* 162 (1976).
24. E. Braunwald. Salvage of ischemic myocardium. In *Pathophysiology and Therapeutics of Myocardial Ischemia.* A. M. Lefer, G. J. Kelliher, and M. J. Rovetto, Eds. Spectrum Publications, New York, 1977, pp. 265–306.
25. R. J. Gryglewski, S. Bunting, S. Moncada, R. J. Flower, and J. R. Vane. Arterial walls are protected against deposition of platelet thrombi by a substance (Prostaglandin X) which they make from prostaglandin endoperoxides. *Prostaglandins, 12,* 685 (1976).
26. R. A. Johnson, D. R. Morton, J. H. Kinner, R. R. Gorman, J. C. McGuire, F. F. Sun, N. Whittaker, S. Bunting, J. Salmon, S. Moncada, and J. R. Vane. The chemical structure of prostaglandin X (Prostacyclin). *Ibid.,* 915.
27. A. M. Lefer, M. L. Ogletree, B. J. Smith, M. J. Silver, K. C. Nicolaou, W. E. Barnette, and G. P. Gasic. Prostacyclin: A potentially valuable agent for preserving myocardial tissue in acute myocardial ischemia. *Science, 200,* 52 (1978).
28. G. T. Raflo, S. L. Wangensteen, T. M. Glenn, and A. M. Lefer. Mechanism of the protective effects of prostaglandins E_1 and $F_{1\alpha}$ in canine endotoxin shock. *Eur. J. Pharmacol., 24,* 88 (1973).
29. T. Takano, J. K. Vayden, H. B. Rose, E. Corday, H. J. C. Swan. Beneficial effects of prostaglandin El in acute myocardial infarction. *Am. J. Cardiol., 39,* 297 (1977).
30. M. L. Ogletree and A. M. Lefer. Prostaglandin-induced preservation of the ischemic myocardium. *Circ. Res., 42,* 218 (1978).
31. H. Yamazaki, M. M. Bodenheimer, V. S. Banka, J. Lewandowski, and R. H. Helfant. The effect of a new prostaglandin PGB, on length-tension relationships following partial coronary occlusion and reperfusion. Submitted to American Heart Association, Dallas, 1978.
32. G. Moss, T. Maglocchetti, and R. Quarmby. Immediate restoration of central nervous system autonomic cardiopulmonary control: Survival of "lethal" cerebral hypoxia by treatment with prostaglandin B_2. *Surg. Forum, 29,* 513 (1978).

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